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Short communication

Mutations in the quinolone-resistance determining region (QRDR) of *Salmonella* strains isolated from pigs in Spain

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Abstract

Quinolone-resistance determining region (QRDR) of *Gyrase* A gene was sequenced in 54 *Salmonella* strains of pig origin that have different quinolone-resistance patterns. Those strains accounted for 12 different serotypes. Mutations at Ser83 or Asp87 were predominant in the studied isolates. However, for serotypes Anatum and Virchow, resistance to quinolones seemed to be linked to specific mutations, namely, Ser83 → Tyr and Ser83 → Phe, respectively. Other mutations found in different positions did not seem to have clinical significance except for changes at Asp82.

Keywords: Quinolone; Resistance; QRDR; *Salmonella*; Pig

1. Introduction

Nowadays, an intense debate on the causes of the increasing antimicrobial resistance is being held among scientists. It has been suggested that the non-therapeutic use of antimicrobial agents in animals is one of the main factors that contribute to the emergence of drug resistances. Particularly, there is a concern for the development of resistant zoonotic bacteria such as *Salmonella* that may pass to people through the food chain and can produce untreatable disease. For example, a *Salmonella* strain of pig origin with decreased susceptibility to quinolones caused a severe outbreak of salmonellosis in Denmark where two people died (Mølbak et al., 1999). Fluoroquinolones have become the first choice treatment for septicemic salmonellosis and thus, the emergence of quinolone-resistant *Salmonella* strains causes a considerable concern.

Acquired resistance to fluoroquinolones arises from several mechanisms: mutations of the target enzymes, modification of membrane porins or overproduction of proteins involved in efflux systems such as the AcrAB-TolC (Everett et al., 1996; Hooper,

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2001a, 2001b; Baucheron et al., 2004). In Gram negative bacteria, quinolone resistance is usually conferred by point mutations in target enzymes, especially those encoding for the DNA gyrase (*gyr*A, *gyr*B, *par*C, *par*E). For *Salmonella*, mutations in the *gyr*A gene, that encodes for the subunit A of the DNA gyrase, seem to be responsible for the development of most resistances to quinolones. These mutations have been clustered in a region of the gene product between amino acids 67 and 122, the so-called quinolone-resistance determining region (QRDR) (Deguchi et al., 1996; Ferrero et al., 1994). Single amino acid changes at Ser-83 (change by Phe, Tyr, or Ala) or at Asp-87 (change by Gly, Asn, or Tyr) are the most frequent (Griggs et al., 1996). However, some authors suggested that this QRDR can be larger and could start at position 51 (Friedman et al., 2001).

In the present study, we investigated the mutations in the QRDR of *Salmonella* isolates of porcine origin in order to figure out their distribution and significance.

2. Materials and methods

Fifty-four *Salmonella* strains were selected from a bacterial collection of pig isolates of Catalonia (Spain) obtained from 1998 to 2002. Selected strains were chosen representing the most frequent pig isolates in Spain for that period. Susceptibility profiles of these isolates have been described before (Mateu et al., 2002; Mejia et al., 2003). Briefly, strains were classified for quinolone resistance according to the results of Kirby–Bauer disk diffusion test (nalidixic acid, and ciprofloxacin) or by the minimal inhibitory concentration determined in a microdilution test (enrofloxacin). All susceptibility tests were done according to NCCLS recommendations (NCCLS, 2002). Fourteen strains were susceptible to all quinolones. The remaining 40 strains were resistant or had decreased susceptibility to 1 or more drugs. For nalidixic acid, 37 strains were resistant (inhibition diameter $\leq$ 13 mm) and 3 were classified as intermediate (14–18 mm). For enrofloxacin 3 strains were resistant (MIC $>$ 2.0 μg/ml) and 31 were intermediate (MIC $>$ 0.5 and $\leq$2.0 μg/ml); for ciprofloxacin only 2 strains were intermediate (16–20 mm) and all others were susceptible. Distribution of isolates by serotype was: Typhimurium (15), Tilburg (9), 4,5,12:i:- (7), Virchow (5), Anatum (4), Brandenburg (4), Hadar (3), Enteritidis (3), and one strain each of serotypes Abony, Bredeney, Grumpensis and Choleraesuis. According to the available data, isolates were epidemiologically unrelated.

Selected isolates were cultured overnight in BHI broth at 37 °C. Then, cultures were centrifuged and suspended in 1 ml of Rnase and Dnase free water (Ambion). DNA extraction was done by boiling for 10 min. Amplification and sequencing of the *gyr*A QRDR region was done according to a previously described PCR protocol (Walker et al., 2001) modified from Griggs et al. (1996). With this technique the amplicon contained a region between codons 37 and 151 of the *gyr*A gene. PCR products were then sequenced using an ABI 373 A Stretch sequencer. Predicted amino acid sequences were compared to a quinolone-susceptible reference strain LT2 (GenBank accession number AE008801)

3. Results

Mutations in the *gyr*A gene were found in 42 strains (77.7%). The most frequent mutation ($n = 23$) corresponded to codon at position 87 (Asp → Asn, 21 strains; Asp → Tyr, two strains), followed by changes at position 83 ($n = 9$) (Ser → Phe, six strains; Ser → Tyr, three strains). Changes Asp87 → Tyr were found only in serotype Enteriditis (two isolates) while all Ser83 → Tyr changes were found only in serotype Anatum (three isolates). Five of the six strains with a change Ser83 → Phe belonged to serotype Virchow. In one case, the recently described mutation at position 82 (Asp → Arg) was found in one isolate of serotype Tilburg (MIC for enrofloxacin = 1.3 μg/ml) that also had a mutation in Asp87 → Asn. None of the strains had a double Ser83/Asp87 mutation.

We also found other mutations in the *gyr*A QRDR that were not described before. These mutations could be found alone or in association with other changes. Thus, 16 strains mutated in position 78 (His → Asn or less commonly His → Asp). Four strains had a Pro79 → His mutation, three a Gly107 → Cys change and two strains had mutations in position 64 (Lys → Asn and Lys → Ser). These changes were found in several serotypes regardless whether or not a given strain was susceptible or resistant to quinolones.